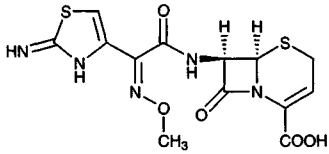
Ceftizoxime



Molecular formula: $C_{13}H_{13}N_5O_5S_2$
Molecular weight: 383.41
CAS Registry No.: 68401-81-0, 68401-82-1 (sodium salt)
Merck Index: 2000
Lednicer No.: 3 218

SAMPLE
Matrix: blood
Sample preparation: After serum deproteination with MeCN, extract the serum samples with 1 mL dichloromethane. Inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:pH 7.0 phosphate buffer 25:75
Flow rate: 1.5
Detector: UV 254

CHROMATOGRAM
Internal standard: acetazolamide
Limit of quantitation: 250 ng/mL

KEY WORDS
serum

REFERENCE
Belliveau,P.P.; Freeman,C.D.; Nicolau,D.P.; Nightingale,C.H.; Tessier,P.R.; Quintiliani,R. Serum bactericidal activity of ceftizoxime and ceftriaxone against pathogens associated with community-acquired and nosocomial pneumonias, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1024–1027.

SAMPLE
Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM
Retention time: 4.3 (mobile phase A), 4.9 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftriaxone, cefuroxime, cephaloridine, cephalothin

Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, teicoplanin, theophylline, vancomycin
Interfering: ranitidine

KEY WORDS
column-switching; serum

REFERENCE
Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 100 μL 200 μg/mL cefotaxime in water + 1 mL MeCN, vortex for 5 s, centrifuge at 30 g for 5 min. Remove the supernatant and add it to 1.5 mL dichloromethane, vortex for 5 s, centrifuge for 5 min, inject a 10-20 μL aliquot of the upper aqueous layer.

HPLC VARIABLES
Guard column: 50 mm long CO:PELL ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:glacial acetic acid 13:84.2:2.8
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 310

CHROMATOGRAM
Retention time: 6
Internal standard: cefotaxime (9)
Limit of quantitation: 1500 ng/mL

OTHER SUBSTANCES
Simultaneous: ceftriaxone, cephalexin, moxalactam, cephaloridine, cefoxitin, cefamandole
Noninterfering: gentamicin, tobramycin, amikacin, clindamycin, erythromycin, vancomycin, penicillin, cefoperazone, piperacillin, ticarcillin, carbenicillin, apalcillin
Interfering: cefazolin, cephapirin

KEY WORDS
serum

REFERENCE
McCormick,E.M.; Echols,R.M.; Rosano,T.G. Liquid chromatographic assay of ceftizoxime in sera of normal and uremic patients, *Antimicrob.Agents Chemother.*, **1984**, *25*, 336–338.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 2 mL MeCN, vortex for 1 min, centrifuge at 4° at 3000 g for 5 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex for 30 s, centrifuge at 3000 g for 5 min, remove the upper aqueous layer and keep it at 4°, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Bondapak C18
Mobile phase: MeCN:100 mM pH 6.1 phosphate buffer 2:98
Injection volume: 20
Detector: UV 229

CHROMATOGRAM
Limit of quantitation: 100 ng/mL

KEY WORDS
serum

REFERENCE
Fortunato,S.J.; Bawdon,R.E.; Welt,S.I.; Swan,K.F. Steady-state cord and amniotic fluid ceftizoxime levels continuously surpass maternal levels, *Am.J.Obstet.Gynecol.*, **1988**, *159*, 570–573.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 500 μL ice-cold 50 μg/mL cefotaxime in MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, hold at -20° for 10 min, centrifuge at 1500 g for 10 min, inject 15 μL of supernatant.

HPLC VARIABLES
Guard column: 10 μm C18 Guard-PAK
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water:glacial acetic acid 100:876:24
Flow rate: 1.5
Injection volume: 15
Detector: UV 254

CHROMATOGRAM
Internal standard: cefotaxime
Limit of detection: 800 ng/mL

OTHER SUBSTANCES
Also analyzed: ceftazidime

KEY WORDS
serum

REFERENCE
Deeter,R.G.; Weinstein,M.P.; Swanson,K.A.; Gross,J.S.; Bailey,L.C. Crossover assessment of serum bactericidal activity and pharmacokinetics of five broad-spectrum cephalosporins in the elderly, *Antimicrob.Agents Chemother.*, **1990**, *34*, 1007–1013.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL 8.5% phosphoric acid. Condition an NH2 SPE cartridge with 1 mL hexane. 500 μL Plasma + 25 μL 8.5% phosphoric acid + 250 μL 1 mg/mL coumarin-3-carboxylic acid in water, add to the C18 SPE cartridge, wash with 500 μL water, wash with 1 mL 8.5% phosphoric acid, wash with 5% MeOH:8.5% phosphoric acid 20:1, elute with 1 mL MeOH: 8.5% phosphoric acid 60:40 into the NH2 SPE cartridge. Wash the NH2 SPE cartridge with 1 mL hexane, wash with 1 mL MeCN, elute with 1 mL water:10% ammonium sulfate 95:5, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 4.6 C18
Mobile phase: Water:2 mM tetramethylammonium hydroxide in MeOH:acetic acid 60:40: 0.5
Flow rate: 0.8
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 5
Internal standard: coumarin-3-carboxylic acid (13)

OTHER SUBSTANCES
Extracted: cefazolin, cefaclor, cephalexin

KEY WORDS
plasma; SPE

REFERENCE
Moore,C.M.; Sato,K.; Hattori,H.; Katsumata,Y. Improved HPLC method for the determination of cephalosporins in human plasma and a new solid-phase extraction procedure for cefazolin and ceftizoxime [letter], *Clin.Chim.Acta*, **1990**, *190*, 121–123.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 20 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Limit of quantitation: 800 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, cephapirin, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cephradine, cefoperazone, cefamandole, cephalothin, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 50-fold with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeCN:20 mM KH_2PO_4 7:93 containing 10 mM triethylamine, adjusted to pH 4.8 with HCl
Flow rate: 1.5
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Simultaneous: ceftazidime, ceftriaxone, metronidazole
Noninterfering: degradation products

KEY WORDS
saline; injections

REFERENCE
Rivers,T.E.; Webster,A.A. Stability of ceftizoxime sodium, ceftriaxone sodium, and ceftazidime with metronidazole in ready-to-use metronidazole bags, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2568–2570.

SAMPLE
Matrix: solutions
Sample preparation: Inject 100 μL onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 × 0.3 5 μm ODS C18 (Nomura); B 150 × 0.3 5 μm ODS C18 (Nomura)
Mobile phase: A 10 mM ammonium acetate adjusted to pH 5 with acetic acid; B MeOH:water:acetic acid 40:60:0.5
Flow rate: A 0.1; B 0.004
Injection volume: 100
Detector: UV 262

CHROMATOGRAM
Retention time: 5.75
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: cefaclor, cephaloridine, cefazolin

KEY WORDS
microbore; column-switching

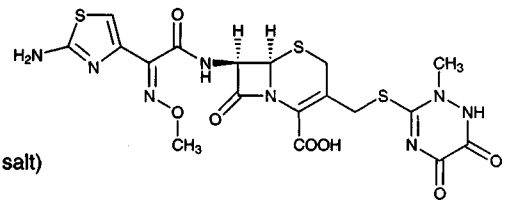
REFERENCE
Moore,C.M.; Sato,K.; Katsumata,Y. High-performance liquid chromatographic determination of cephalosporin antibiotics using 0.3 mm I.D. columns, *J.Chromatogr.*, **1991**, *539*, 215–220.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 5:95
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 240

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

Ceftriaxone



Molecular formula: $C_{18}H_{18}N_8O_7S_3$
Molecular weight: 554.59
CAS Registry No.: 73384-59-5, 104376-79-6 (sodium salt)
Merck Index: 2001

SAMPLE

Matrix: blood
Sample preparation: After serum deproteination with MeCN, extract the serum samples with 1 mL dichloromethane. Inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: MeCN:pH 7.0 phosphate buffer 25:75
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM

Internal standard: o-anisic acid
Limit of quantitation: 10 μg/mL

KEY WORDS

serum

REFERENCE

Belliveau,P.P.; Freeman,C.D.; Nicolau,D.P.; Nightingale,C.H.; Tessier,P.R.; Quintiliani,R. Serum bactericidal activity of ceftizoxime and ceftriaxone against pathogens associated with community-acquired and nosocomial pneumonias, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1024–1027.

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM

Retention time: 10.8 (mobile phase A), 12.6 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftizoxime, cefuroxime, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS
column-switching; serum

REFERENCE
Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE
Matrix: blood
Sample preparation: Dilute serum 1:10 with cold MeOH or filter (Millipore Ultraspec-MC, molecular weight limit 10000), inject an aliquot.

HPLC VARIABLES
Column: 25 × 4.6 5 μm C18
Mobile phase: MeCN:1 M pH 7 phosphate buffer:water 50:1:49 containing 3 g/L hexadecyltrimethylammonium bromide
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 6-7
Limit of quantitation: 500 ng/mL (filtered sample)

KEY WORDS
serum; ultrafiltrate; pharmacokinetics

REFERENCE
Hayward,C.J.; Nafziger,A.N.; Kohlhepp,S.J.; Bertino,J.S.,Jr. Investigation of bioequivalence and tolerability of intramuscular ceftriaxone injections using 1% lidocaine, buffered lidocaine, and sterile water diluents, *Antimicrob.Agents Chemother.*, **1996**, *40*, 485–487.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 245.2

CHROMATOGRAM
Retention time: 5.34

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Reconstitute ceftriaxone powder with water to a drug concentration of 100 mg/mL. Dilute 10 μL 100 mg/mL ceftriaxone in water and 10 μL 100 mg/mL ceftazidime in water to 5 mL with water, inject a 10 μL aliquot of the solution.

HPLC VARIABLES
Column: 100 × 4.6 4 μm Novapak C18
Mobile phase: MeCN:7.5 mM pH 7.0 phosphate buffer 40:60 containing 2 g/L tetrabutylammonium phosphate
Flow rate: 1.5
Injection volume: 10
Detector: UV 242

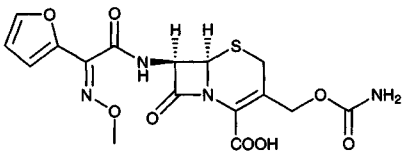
CHROMATOGRAM
Retention time: 2.3
Internal standard: ceftazidime (1.6)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
Plumridge,R.J.; Rieck,A.M.; Annus,T.P.; Langton,S.R. Stability of ceftriaxone sodium in polypropylene syringes at -20, 4, and 20°C, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 2320–2323.

Cefuroxime



Molecular formula: $C_{16}H_{16}N_4O_8S$
Molecular weight: 424.39
CAS Registry No.: 55268-75-2, 56238-63-2 (sodium salt), 64544-07-6 (axetil), 100680-33-9 (pivoxetil)
Merck Index: 2002
Lednicer No.: 3 216

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 267

CHROMATOGRAM

Retention time: 5.3 (mobile phase A), 6.4 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftizoxime, ceftriaxone, cephaloridine, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS

column-switching; serum

REFERENCE

Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 278.3

CHROMATOGRAM
Retention time: 16.293 (peak 1), 16.552 (peak 2)

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bronchoalveolar lavage fluid
Sample preparation: Condition a C18 SPE cartridge with 2 mL MeCN, 1 mL water, and 1 mL 50 mM pH 6.0 citrate buffer. Centrifuge bronchoalveolar lavage fluid at 1150 g and 4° for 10 min, dilute 200-400 μL of the supernatant with 1 mL 50 mM pH 6.0 citrate buffer and add 30 μL 1 mg/mL cephaloridine in 20 mM pH 6.0 phosphate buffer. Add to the SPE cartridge, wash with 1 mL 50 mM pH 6.0 citrate buffer, 500 μL water, two 400 μL portions of MTBE, and three 1 mL portions of MeCN. Dry the cartridge between each washing under full vacuum for at least 1 min. Elute with 1 mL MeOH, evaporate the eluate under nitrogen at 40°, dissolve the residue in 55 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 5 μm Symmetry C18 Sentry (Waters)
Column: 150 × 3.9 5 μm 100 Å Symmetry C18 (Waters)
Mobile phase: MeCN:50 mM pH 3.2 ammonium phosphate buffer 15:85
Flow rate: 0.8
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Retention time: 9.17
Internal standard: cephaloridine (6.25)
Limit of detection: 1 ng/mL
Limit of quantitation: 5 ng/mL

KEY WORDS
SPE

REFERENCE
Rosseel,M.T.; Peleman,R.; Van Hoorbeke,H.; Pauwels,R.A. Measurement of cefuroxime in human bronchoalveolar lavage fluid by high-performance liquid chromatography after solid-phase extraction, *J.Chromatogr.B*, **1997**, *689*, 438–441.

SAMPLE
Matrix: milk

Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 19.5 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 18:82 (Buffer was 20 mM phosphoric acid containing 10 mM sodium decanesulfonate.)
Flow rate: 1
Injection volume: 200
Detector: UV 260

REFERENCE

Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM

Retention time: 2.2
Limit of detection: 60 nM

OTHER SUBSTANCES

Simultaneous: cefoperazone, cefoxitin, ceftazidime, cephalexin, cephradine

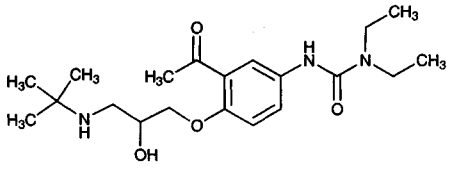
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

Celiprolol



Molecular formula: $C_{20}H_{33}N_3O_4$
Molecular weight: 379.50
CAS Registry No.: 56980-93-9, 57470-78-7 (HCl)
Merck Index: 2007
Lednicer No.: 4 27

SAMPLE

Matrix: blood
Sample preparation: Add 1.5 mL MeCN to 500 µL serum, centrifuge, evaporate the supernatant to dryness, redissolve the residue in 200 µL water. Inject onto column A, wash with MeCN:water 10:90 or MeOH:water 20:80 for 20 min, backflush the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 25 × 4 25 µm pore diameter 6 nm LiChrospher RP-18 ADS (Merck); B 125 × 4 5 µm endcapped LiChroCART HPLC-cartridge RP-18 (Merck)
Mobile phase: MeCN:50 mM pH 4 K_3PO_4 buffer 27:73
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 242, UV 230

CHROMATOGRAM

Retention time: 3.6

OTHER SUBSTANCES

Extracted: metoprolol, tiracizine, talinolol, oxprenolol, metabolites

KEY WORDS

serum; column-switching

REFERENCE

Oertel,R.; Richter,K.; Gramatté,T.; Kirch,W. Determination of drugs in biological fluids by high-performance liquid chromatography with on-line sample processing, *J.Chromatogr.A*, **1998**, *797*, 203–209.

SAMPLE

Matrix: blood
Sample preparation: Add 50 µL 1.4 µg/mL acebutolol, 1 mL 67 mM pH 7.4 phosphate buffer, 200 µL 1 M NaOH, and 6 mL MTBE to 1 mL plasma. Shake for 10 min, centrifuge at 1300 g at 4° for 5 min, freeze the aqueous layer in acetone/dry ice. Add 200 µL 10 mM HCl to the organic layer, shake for 10 min, centrifuge at 1300 g for 5 min. Freeze the aqueous layer, discard the organic phase, eliminate traces of the organic layer using a stream of cold air over the aqueous layer for 3-4 min. Thaw the aqueous layer, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Spherisorb hexyl
Mobile phase: MeCN:15 mM pH 3.5 KH_2PO_4 containing 0.05% triethylamine 45:55
Flow rate: 1
Injection volume: 20
Detector: UV 238

CHROMATOGRAM
Retention time: 4.2
Internal standard: acebutolol (3.3)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma

REFERENCE
Verbesselt,R.; Zugravu,A.; Tjandramaga,T.B.; De Schepper,P.J. Liquid chromatographic determination of total celiprolol or (S)-celiprolol and (R)-celiprolol simultaneously in human plasma, *J.Chromatogr.B*, **1996**, *683*, 231–236.

SAMPLE
Matrix: blood
Sample preparation: Add 50 μL 1.4 μg/mL acebutolol, 1 mL 67 mM pH 7.4 phosphate buffer, 200 μL 1 M NaOH, and 6 mL MTBE to 1 mL plasma. Shake for 10 min, centrifuge at 1300 g at 4° for 5 min, freeze the aqueous layer in acetone/dry ice. Evaporate the organic layer to dryness with a stream of air at 40°. Add 100 μL 0.005% R(-)-1-(1-naphtyl)ethyl isocyanate to the dry residue, vortex, let stand stoppered for 30 min, evaporate with a stream of air at 35°, redissolve the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil BDS C18
Mobile phase: MeCN:15 mM pH 3.5 KH_2PO_4 buffer containing 0.05% triethylamine 50:50
Flow rate: 1
Injection volume: 20
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 16.6 (R), 18 (S)
Internal standard: acebutolol (11)
Limit of quantitation: 2.5 ng/mL

KEY WORDS
plasma; chiral; derivatization

REFERENCE
Verbesselt,R.; Zugravu,A.; Tjandramaga,T.B.; De Schepper,P.J. Liquid chromatographic determination of total celiprolol or (S)-celiprolol and (R)-celiprolol simultaneously in human plasma, *J.Chromatogr.B*, **1996**, *683*, 231–236.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL 200 mg RP 18 SPE cartridge with 3 mL MeOH and 3 mL water. 1 mL Plasma + 50 ng propranolol + 1 mL pH 9 borate buffer, add to SPE cartridge, wash with 5 mL pH 3.15 phosphate buffer, wash with 3 mL water, wash with 500 μL MeCN:water:phosphate buffer 62:32:6, elute with 1 mL MeCN:water:phosphate buffer 62:32:6, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS
Mobile phase: MeCN:water:phosphate buffer 62:32:6
Flow rate: 1
Injection volume: 100
Detector: F ex 335 em 480 (celiprolol) or ex 290 em 350 (propranolol)

CHROMATOGRAM
Retention time: 9
Internal standard: propranolol (12)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
plasma; SPE

REFERENCE
Rostock,G.; Günzel,R.; Glöckl,D. Solid-phase extraction and direct high-performance liquid chromatographic determination of celiprolol in plasma, *Int.J.Clin.Pharmacol.Ther.Toxicol.*, **1992**, *30*, 512–513.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut 40 μm cyanopropylsilica SPE cartridge with 1 mL MeOH at 6 mL/min and with 1 mL pH 7.4 buffer at 6 mL/min. Centrifuge plasma, add 1 mL plasma at 0.18 mL/min to the SPE cartridge, wash with 1 mL pH 7.4 buffer at 1.5 mL/min, elute with 250 μL MeOH:2-aminoheptane 99.8:0.2 at 1.5 mL/min, pass 750 μL pH 3.0 buffer through the cartridge at 1.5 mL/min. Mix both eluates, inject a 250 μL aliquot. (pH 7.4 Buffer was 250 mL 100 mM KH_2PO_4 and 195.5 mL 100 mM NaOH, made up to 1 L, if necessary pH adjusted to 7.4. pH 3.0 Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 4 μm Superspher 100 RP-18 (Merck)
Mobile phase: MeCN:buffer 25:75 containing 0.5% 2-aminoheptane (Buffer was 4 g NaOH in 700 mL water, pH adjusted to 3.0 with 85% phosphoric acid, made up to 1 L with water.)
Column temperature: 37
Flow rate: 1.2
Injection volume: 250
Detector: F ex 350 em 480

KEY WORDS
plasma; SPE

REFERENCE
Hubert,P.; Chiap,P.; Moors,M.; Bourguignon,B.; Massart,D.L.; Crommen,J. Knowledge-based system for the automated solid-phase extraction of basic drugs fom plasma coupled with their liquid chromatographic determination. Application to the biodetermination of β-receptor bocking agents, *J.Chromatogr.A*, **1994**, *665*, 87–99.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 40 μL 20 μg/mL deacetyldiltiazem in water + 1 mL pH 10 borate buffer + 0.5 g NaCl, vortex for 30 s, add 6 mL hexane:isopropanol 95:5, vortex for 30 s, shake for 10 min, centrifuge at 700 g for 10 min. Remove the organic layer and add it to 200 μL 5 mM sulfuric acid, vortex for 30 s, shake for 10 min, centrifuge at 700 g for 5 min, inject a 40 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:MeOH:buffer 22.5:22.5:55 (Buffer was 7.5 g sodium acetate trihydrate + 0.6 g 1-heptanesulfonic acid in 1 L water, adjust pH to 4.5 with acetic acid.)
Flow rate: 1.5
Injection volume: 40
Detector: UV 237

CHROMATOGRAM

Retention time: 3.2
Internal standard: deacetyldiltiazem (7)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Simultaneous: verapamil, propranolol, diltiazem, desipramine
Noninterfering: atenolol, aspirin, caffeine, ibuprofen, lidocaine, metoprolol, nifedipine

KEY WORDS

plasma

REFERENCE

Rutledge,D.R.; Abadi,A.H.; Lopez,L.M. Simultaneous determination of verapamil and celiprolol in human plasma, *J.Chromatogr.Sci.*, **1994**, *32*, 153–156.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 233

CHROMATOGRAM

Retention time: 4.36
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ke-

tamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 1 mL Plasma or urine + 0.1 (plasma) or 1 (urine) mL 1 μg/mL (R)-(+)-propranolol in buffer + 5 mL dichloromethane:isopropanol 95:5, shake for 15 min, centrifuge at 10° at 2500 g for 20 min. Remove 4 mL of the organic layer, add 4 mL dichloromethane:isopropanol 95:5 to the aqueous layer, shake for 15 min, centrifuge at 10° at 2500 g for 20 min. Combine the organic layers and evaporate them to dryness in a vacuum centrifuge at 30°, reconstitute the residue in 250 μL mobile phase. (Buffer was 3.092 g boric acid, 3.728 g KCl, and 1.756 g NaOH in 1 L water, pH 10.0.)

HPLC VARIABLES

Guard column: 50 × 4.6 10 μm Chiralcel OD
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: n-Hexane:isopropanol:diethylamine 85:20:0.1
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 480 (celiprolol) or ex 295 em 345 (propranolol)

CHROMATOGRAM

Retention time: 12.7 (S)-(-), 17.0 (R)-(+)
Internal standard: (R)-(+)-propranolol (9.5)
Limit of detection: 2.5 ng/mL (urine), 1.5 ng/mL (plasma)

OTHER SUBSTANCES

Noninterfering: metabolites, acebutolol, amiloride, atenolol, captopril, carteolol, clonidine, dihydralazine, furosemide, hydrochlorothiazide, labetalol, α-methyldopa, metoprolol, nifedipine, oxprenolol, penbutolol, pindolol, reserpine, triamterene, verapamil

KEY WORDS
plasma; chiral; pharmacokinetics

REFERENCE
Hartmann,C.; Krauss,D.; Spahn,H.; Mutschler,E. Simultaneous determination of (*R*)- and (*S*)-celiprolol in human plasma and urine: high-performance liquid chromatographic assay on a chiral stationary phase with fluorimetric detection, *J.Chromatogr.*, **1989**, *496*, 387–396.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 *g* for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 232.2

CHROMATOGRAM
Retention time: 11.493

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 30:70 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 2.86 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)
Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 5.25

OTHER SUBSTANCES
Also analyzed: acebutolol, bunitrolol, carazolol, esmolol, mepindolol, metoprolol, timolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions

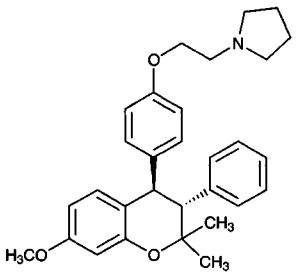
HPLC VARIABLES
Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 5.29

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE
Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Centchroman



Molecular formula: $C_{30}H_{35}NO_3$
Molecular weight: 457.61
CAS Registry No.: 78994-24-8, 51023-56-4 (HCl)
Merck Index: 2018

SAMPLE

Matrix: blood
Sample preparation: 1-2 mL Serum + 3 mL diethyl ether, vortex for 15 s, centrifuge at 1700 rpm for 5 min, freeze in liquid nitrogen, remove the organic layer. Extract twice more and combine the organic layers, evaporate them to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 10 μm cyano guard column (Kontron)
Column: 110 × 4.6 5 μm Spherisorb cyano
Mobile phase: MeCN:10 mM KH_2PO_4:0.3 mM orthophosphoric acid 60:32:8
Flow rate: 1
Detector: F ex 279 em 305

CHROMATOGRAM

Retention time: 6.2
Limit of detection: 2 ng/mL

KEY WORDS

serum; pharmacokinetics

REFERENCE

Paliwal,J.K.; Gupta,R.C.; Grover,P.K.; Asthana,O.P.; Srivastava,J.S.; NityaNand,S. High performance liquid chromatographic (HPLC) determination of centchroman in human serum and application to single-dose pharmacokinetics, *Pharm.Res.*, **1989**, *6*, 1048–1051.

SAMPLE

Matrix: blood, milk
Sample preparation: Serum. 500 μL Serum + 50 μL 1% KOH, vortex for 15 s, add 3 mL diethyl ether, vortex for 1 min, centrifuge at 100 g for 10 min, freeze in liquid nitrogen, remove the organic layer, repeat the extraction of the aqueous layer. Combine the organic layers and evaporate them to dryness under reduced pressure, reconstitute the residue in 100 μL mobile phase, inject an 50 μL aliquot. Milk. 500 μL Milk + 2 mL MeCN, vortex for 1 min, let stand at 4° for 30 min, centrifuge at 1000 g for 10 min. Remove the supernatant and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL 100 mM pH 8.7 K_2HPO_4, add 3 mL diethyl ether, vortex for 1 min, centrifuge at 100 g for 10 min, freeze in liquid nitrogen, remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 100 μL mobile phase, inject an 50 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Spheri-5 cyano
Column: 100 × 4.6 5 μm Spheri-5 cyano
Mobile phase: MeCN:buffer 58:42 (Buffer was 20 mM KH_2PO_4 adjusted to pH 3 with 20% orthophosphoric acid.)
Flow rate: 1.5

Injection volume: 50
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 5.6
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Lal,J.; Paliwal,J.K.; Grover,P.K.; Gupta,R.C. Simultaneous liquid chromatographic determination of centchroman and its 7-demethylated metabolite in serum and milk, *J.Chromatogr.B*, **1994**, *658*, 193–197.

SAMPLE
Matrix: formulations
Sample preparation: Finely powder 20 tablets, weigh out amount equivalent to 10 mg centchroman hydrochloride, extract three times with 5 mL portions of MeOH, combine the extracts, centrifuge, make up to 25 mL with MeOH, remove a 250 μL aliquot and make it up to 25 mL with mobile phase.

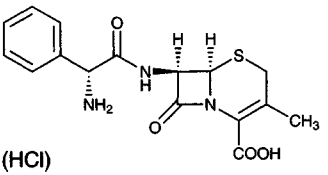
HPLC VARIABLES
Column: 250 × 4.6 5 μm Phenomenex ODS
Mobile phase: MeCN:water:10% tetramethylammonium hydroxide in water 80:20:0.4, adjusted to pH 7.6 with 100 mM orthophosphoric acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 24.0 (trans), 34.0 (cis)
Limit of detection: 100 ng (trans), 50 ng (cis)
Limit of quantitation: 180 ng (trans), 110 ng (cis)

KEY WORDS
tablets; bulk

REFERENCE
Dwivedi,A.K.; Sirkar,K.P.; Bhatt,G.R.; Seth,R.K.; Singh,S.; Sarin,J.P.S. Determination of *cis*- and *trans*-centchroman in its dosage forms by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *663*, 187–190.

Cephalexin



Molecular formula: $C_{16}H_{17}N_3O_4S$
Molecular weight: 347.39
CAS Registry No.: 15686-71-2, 23325-78-2 (monohydrate), 105879-42-3 (HCl)
Merck Index: 2021
Lednicer No.: 1 417; 2 439; 4 182

SAMPLE

Matrix: blood
Sample preparation: Mix 10 mL serum with an equal volume MeCN, vortex for 20 s, centrifuge at 2040 g for 10 min. Dilute the supernatant to 50 mL with water. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 100 × 8 4 μm Nova-Pak C18 Radial-Pak
Mobile phase: Gradient. A was MeCN:MeOH:10 mM pH 4.7 acetate buffer 2:11:87. B was MeCN:MeOH:10 mM pH 4.7 acetate buffer 11:2:87. A:B 100:0 for 5 min, to 70:30 over 10 min, to 0:100 over 12 min
Flow rate: 1
Injection volume: 50
Detector: E, pulsed electrochemical detector with Ag/AgCl reference electrode, stainless steel counter electrode and circular gold working electrode using indirect pulsed amperometric detection (PAD) or integrated pulsed amperometric detection (IPAD)

CHROMATOGRAM

Retention time: 4
Limit of detection: 30 ppb (indirect PAD), 10 ppb (integrated PAD)

OTHER SUBSTANCES

Simultaneous: cefaclor, cefadroxil, cefazolin, cefotaxime, cefoxitin, cefsulodin, cefuroxime, cephaloglycin, cephalothin, cephradine

KEY WORDS

pig; serum

REFERENCE

Yun,E.K.; Prince,A.J.; McMillin,J.E.; Welch,L.E. High-performance liquid chromatographic separation and electrochemical detection of cephalosporins, *J.Chromatogr.B*, **1998**, *712*, 145–152.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 100 μL water + 50 μL 25 μg/mL cefprozil + 100 μL 1 M hydrochloric acid, vortex briefly. Filter (Centrifree micropartition unit) 1 mL mixture while centrifuging at 2000 g for 10 min. Inject a 25 μL aliquot of the ultrafiltrate. Urine. 250 μL urine + 500 μL 250 μg/mL cefprozil + 4.25 mL 10 mM pH 3.5 acetate buffer, vortex briefly. Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleogil C18 (plasma) or 125 × 4.6 5 μm Lichrosorb C18 (urine)
Mobile phase: MeCN:10 mM phosphoric acid (adjusted to pH 3.8 with NaOH) 8:92 (plasma) or MeCN:10 mM phosphoric acid (adjusted to pH 3.8 with NaOH) 10:90 (urine)
Flow rate: 1.2 (plasma), 1.0 (urine)
Injection volume: 25
Detector: UV 260

CHROMATOGRAM
Retention time: 12 (plasma), 9.7 (urine)
Internal standard: cefprozil (9.2) (plasma), (6.3) (urine)
Limit of quantitation: 200 ng/mL (plasma), 10 μg/mL (urine)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Barbhaiya,R.H. A pharmacokinetic comparison of cefadroxil and cephalexin after administration of 250, 500 and 1000 mg solution doses, *Biopharm.Drug Dispos.*, **1996**, *17*, 319–330.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.875 (peak 1), 4.792 (peak 2)

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: milk, tissue
Sample preparation: Milk. Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 18.5 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. Tissue. Blend 5

g tissue, 5 mL water, 2 mL 100 mM tetraethylammonium chloride (for liver and kidney 1 mL 200 mM tetraethylammonium chloride and 1 mL 5 mM KH_2PO_4), and 40 mL MeCN at half power for 1 min, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate (20 mL for liver and kidney), add 2 mL buffer, add 5 mL water, add 5 mL t-butanol, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Proceed as above. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer A 35:65 (milk) or MeCN:buffer B 33:67 (tissue) (Buffer A was 10 mM phosphoric acid containing 5 mM potassium dihydrogen phosphate and 5 mM sodium dodecyl sulfate. Buffer B was 6.7 mM phosphoric acid containing 3.3 mM potassium dihydrogen phosphate and 2.5 mM sodium dodecyl sulfate.)
Flow rate: 1
Injection volume: 200
Detector: UV 260

KEY WORDS
muscle; liver; kidney

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:30 mM pH 7.0 sodium phosphate buffer 15:85
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 Lichrospher 100 RP-18
Mobile phase: MeOH:2.5 mM pH 5.6 sodium phosphate buffer 18:80
Flow rate: 1
Injection volume: 20
Detector: UV 274

CHROMATOGRAM
Retention time: 4
Limit of detection: 60 nM

OTHER SUBSTANCES
Simultaneous: cefoperazone, cefoxitin, cefuroxime, ceftazidime, cephradine

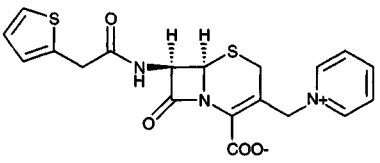
KEY WORDS

comparison with capillary electrophoresis

REFERENCE

Choi,O.-K.; Song,Y.-S. Determination of cefuroxim levels in human serum by micellar electrokinetic capillary chromatography with direct sample injection, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1265–1270.

Cephaloridine



Molecular formula: $C_{19}H_{17}N_3O_4S_2$
Molecular weight: 415.49
CAS Registry No.: 50-59-9
Merck Index: 2025
Lednicer No.: 1 417

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase A or B, elute with mobile phase A or B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: A MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate; B MeOH:10 mM pH 7.0 phosphate buffer 30:70 containing 5 mM tetrabutylammonium hydrogen sulfate
Flow rate: 1
Injection volume: 20
Detector: UV 252

CHROMATOGRAM

Retention time: 4.0 (mobile phase A), 4.7 (mobile phase B)
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES

Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftizoxime, ceftriaxone, cefuroxime, cephalothin
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS

column-switching; serum

REFERENCE

Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE

Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 11.52:0.48:88, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5
Internal standard: cefoperazone (7.5)
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Extracted: cefotaxime
Interfering: cefoxitin, cephalexin

KEY WORDS
plasma

REFERENCE
Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE
Matrix: blood
Sample preparation: Mix 100 μL plasma + 300 μL 5 μg/mL cefotaxime in pH 3.5 10 mM acetate buffer and keep at 4°. Inject 100 μL onto column A with mobile phase A. After 5 min backflush column A with mobile phase B onto column B for 3 min. Re-equilibrate column A with mobile phase A for 16 min.

HPLC VARIABLES
Column: A 40 × 2 37-50 μm Corasil RP C18; B 20 × 4 25-40 μm Lichrosorb RP-8 + 250 × 4 Partisil ODS-3
Mobile phase: A 10 mM pH 3.5 acetate buffer; B MeCN:20 mM pH 4.3 acetate buffer 15:85
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 10.3
Internal standard: cefotaxime
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: cephalexin, cefoxitin, cefuroxime
Noninterfering: cefotiam, cefadroxil, cefazolin, cefoperazone, cephalothin, cefamandole, aspirin, diclofenac, alclofenac, lonazolac, piroxicam, ibuprofen, indomethacin, ketoprofen, naproxen, phenylbutazone, mefenamic acid, caffeine

KEY WORDS
plasma; column-switching; rat; human

REFERENCE
Lee,Y.J.; Lee,H.S. Simultaneous determination of cefoxitin, cefuroxime, cephalexin and cephaloridine in plasma using HPLC and a column-switching technique, *Chromatographia*, **1990**, *30*, 80–84.

SAMPLE
Matrix: blood, CSF, gastric contents, urine
Sample preparation: 200 μL Serum, urine, CSF, or gastric fluid + 300 μL reagent. Flush column A to waste with 500 μL 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500 μL 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

HPLC VARIABLES

Column: A 40 μm preparative grade C18 (Analytichem); B 75 × 2.1 pellicular C18 (Whatman) + 250 × 4.6 5 μm C8 end-capped (Whatman)
Mobile phase: Gradient. A was 50 mM pH 4.5 KH_2PO_4. B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.
Column temperature: 50
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM

Retention time: 9 (broad peak)
Internal standard: heptanophenone (19)

OTHER SUBSTANCES

Extracted: acetaminophen, allobarbital, azinphos, barbital, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, thioridazine, trimethoprim

KEY WORDS

serum; column-switching

REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *612*, 191–198.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 20 × 3.9 5 μm Symmetry C18 Sentry (Waters)
Column: 150 × 3.9 5 μm 100 Å Symmetry C18 (Waters)
Mobile phase: MeCN:50 mM pH 3.2 ammonium phosphate buffer 15:85
Flow rate: 0.8
Injection volume: 40
Detector: UV 280

CHROMATOGRAM

Retention time: 6.25

OTHER SUBSTANCES

Simultaneous: cefuroxime

REFERENCE

Rosseel,M.T.; Peleman,R.; Van Hoorbeke,H.; Pauwels,R.A. Measurement of cefuroxime in human bronchoalveolar lavage fluid by high-performance liquid chromatography after solid-phase extraction, *J.Chromatogr.B*, **1997**, *689*, 438–441.

SAMPLE

Matrix: solutions

Sample preparation: Inject 100 μL onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B, elute column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 30 × 0.3 5 μm ODS C18 (Nomura); B 150 × 0.3 5 μm ODS C18 (Nomura)
Mobile phase: A 10 mM ammonium acetate adjusted to pH 5 with acetic acid; B MeOH: water:acetic acid 40:60:0.5
Flow rate: A 0.1; B 0.004
Injection volume: 100
Detector: UV 262

CHROMATOGRAM

Retention time: 7.03
Limit of detection: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: cefaclor, cefazolin, ceftizoxime

KEY WORDS

microbore; column-switching

REFERENCE

Moore,C.M.; Sato,K.; Katsumata,Y. High-performance liquid chromatographic determination of cephalosporin antibiotics using 0.3 mm I.D. columns, *J.Chromatogr.*, **1991**, *539*, 215–220.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 220 × 4.6 Spheri 5 ODS-224
Mobile phase: 100 mM sodium dodecyl sulfate, pH 6.72
Flow rate: 1
Injection volume: 10
Detector: UV 260

CHROMATOGRAM

Retention time: 6.5

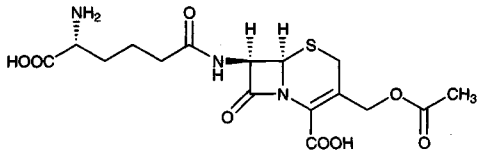
OTHER SUBSTANCES

Simultaneous: cefazolin, cephalothin, cephalexin, cephradine, 7-aminocephalorospanic acid, 7-aminodesacetoxycephalosporanic acid

REFERENCE

Garcia Pinto,C.; Pérez Pavón,J.L.; Moreno Cordero,B. Micellar liquid chromatography of zwitterions: Retention mechanism of cephalosporins, *Analyst*, **1995**, *120*, 53–62.

Cephalosporin C



Molecular formula: $C_{16}H_{21}N_3O_8S$
Molecular weight: 415.42
CAS Registry No.: 61-24-5
Merck Index: 2026

SAMPLE

Matrix: solutions
Sample preparation: Dissolve sample in mobile phase to a concentration of about 1 mg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm β-CyD (Advanced Separation Technologies Inc., USA)
Mobile phase: MeOH:buffer 42:58 (Buffer was 5 mM tetraethylammonium acetate adjusted to pH 3.6 with glacial acetic acid.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM

Retention time: 31

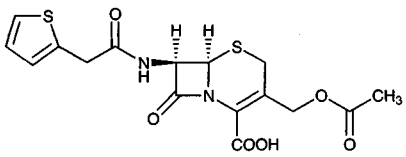
OTHER SUBSTANCES

Simultaneous: 7-ACA, 7-ADCA, cefaclor, cefaloridine, cefazolin, cefoperazone, cefotaxime, ceftazidime

REFERENCE

Tsou,T.-L.; Wu,J.-R.; Wang,T.-M. The effects of separation of cephalosporins by HPLC with β-cyclodextrin bonded stationary phase, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1081–1095.

Cephalothin



Molecular formula: $C_{16}H_{16}N_2O_6S_2$
Molecular weight: 396.44
CAS Registry No.: 153-61-7, 58-71-9 (sodium salt)
Merck Index: 2028
Lednicer No.: 1 420

SAMPLE

Matrix: aqueous humor
Sample preparation: 150 μL Aqueous humor + 50 μL 400 mM HCl, mix, add 700 μL chloroform:1-pentanol 3:1, mix by swirl-mixing for 5 min, centrifuge at 300 g for 5 min, discard the organic layer. Centrifuge the aqueous layer briefly, hold it at 4°, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.5 5 μm Ultrasphere RP-ODS
Mobile phase: MeOH:water:buffer 40:48:12, the final pH was adjusted to 6.7 with triethylamine (Buffer was 50 mM pH 6.7 morpholinopropanesulfonic acid (MOPS)-triethylamine.)
Column temperature: 32
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.74
Internal standard: cephalothin

OTHER SUBSTANCES

Simultaneous: piperacillin, carbenicillin, mezlocillin, azlocillin, cefamandole, cefoxitin, cefuroxime, scopolamine, sulfamethoxazole, theophylline, ticarcillin, timolol
Noninterfering: acetazolamide, amitriptyline, atropine, carbachol, cefazolin, cefoperazone, cefotaxime, chlorpheniramine, codeine, diazepam, echothiophate, epinephryl borate, imipramine, prednisolone acetate, tropicamide, xylazine
Interfering: acetaminophen, ampicillin, caffeine, salicylic acid

KEY WORDS

rabbit; cephalothin is IS

REFERENCE

Riegel,M.A.; Ellis,P.P. High-performance liquid chromatographic assay for piperacillin in aqueous humor of the eye, *J.Chromatogr.*, **1988**, *424*, 177–181.

SAMPLE

Matrix: blood
Sample preparation: Dilute serum with an equal volume of water, inject a 20 μL aliquot onto column A, elute column A to waste with MeOH:10 mM pH 7.0 phosphate buffer 5:95 at 0.3 mL/min, after 1.3 min elute the contents of column A onto column B with mobile phase, elute with mobile phase, monitor the effluent from column B.

HPLC VARIABLES

Column: A 50 × 2.1 40 μm Supelclean LC-NH_2; B 150 × 4.6 3 μm Supelcosil LC-18
Mobile phase: MeCN:MeOH:10 mM pH 7.0 phosphate buffer 15:20:65 containing 5 mM tetrabutylammonium hydrogen sulfate

Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 13.0
Limit of detection: 500-2000 ng/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefazolin, cefodizime, cefoperazone, cefoxitin, ceftizoxime, ceftriaxone, cephaloridine, cefuroxime
Noninterfering: acetaminophen, acyclovir, digoxin, fluconazole, ranitidine, teicoplanin, theophylline, vancomycin

KEY WORDS
column-switching; serum

REFERENCE
Bompadre,S.; Ferrante,L.; Leone,L. On-line solid-phase extraction of cephalosporins, *J.Chromatogr.A*, **1998**, *812*, 191–196.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 19.2:0.8:20:80, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: cefamandole (4)
Limit of detection: 500 ng/mL

KEY WORDS
plasma

REFERENCE
Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Place 500 μL serum in an ultrafree MC filter unit with a 10000 MW cutoff (Millipore), centrifuge at 16000 g for 30 min, inject 180 μL a aliquot of the filtrate. Tissue. Homogenize 100 mg tissue in 1 mL water using a Polytron homogenizer (Brinkman), centrifuge at 1000 g for 15 min, filter (Acrodisc CR PTFE 0.2 μm filter, prewet with water and MeOH) the supernatant, inject a 180 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: μBondapak C18 Guard-Pak

Column: 100 × 8 10 μm μBondapak C18
Mobile phase: MeCN:5 mM KH_2PO_4:glacial acetic acid 22:77.5:0.5
Flow rate: 2
Injection volume: 180
Detector: UV 235

CHROMATOGRAM
Retention time: 13.55 (serum), 13.95 (tissue)
Internal standard: cephalothin

OTHER SUBSTANCES
Extracted: cefoxitin

KEY WORDS
cat; colon; serum; cephalothin is IS

REFERENCE
Cox,S.K.; Burnette,J.D.; Huss,B.T.; Frazier,D. Determination of cefoxitin in serum and tissue, *J.Chromatogr.B*, **1998**, *705*, 145–148.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Dilute plasma with three volumes of MeOH, centrifuge at 2000 rpm for 10 min, filter (0.45 μm) the supernatant, inject a 10 μL aliquot. Urine. Dilute urine with 0.1% phosphate buffer, centrifuge at 3000 rpm for 15 min, filter (0.45 μm) the supernatant, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Zorbax ODS
Mobile phase: MeOH:0.2% ammonium acetate 40:60
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; rabbit; human; pharmacokinetics

REFERENCE
Sakata,Y. The pharmacokinetic studies of cephalothin, cefazolin and cefmetazole in the neonates and the premature babies, *Kurume Med.J.*, **1980**, *27*, 275–298.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 30 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 30
Limit of quantitation: 2370 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, cephapirin, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cephradine, cefoperazone, cefamandole, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: cell suspensions
Sample preparation: Add two volumes cold MeOH, centrifuge at 7080 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.5 μBondapak C18
Mobile phase: Gradient. MeOH:10 mM pH 6.8 K_2HPO_4 from 20:80 to 60:40 over 20 min
Flow rate: 1
Detector: UV 254

OTHER SUBSTANCES
Extracted: metabolites, cephaloglycin

KEY WORDS
rabbit; liver; kidney

REFERENCE
Williams,P.D.; Laska,D.A.; Tay,L.K.; Hottendorf,G.H. Comparative toxicities of cephalosporin antibiotics in a rabbit kidney cell line (LLC-RK_1), *Antimicrob.Agents Chemother.*, **1988**, *32*, 314–318.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water (if necessary), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak phenyl
Mobile phase: MeOH:water 30:70 containing 10 mM ammonium acetate
Flow rate: 2.2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Simultaneous: cefazolin

KEY WORDS
saline; 5% dextrose; stability-indicating

REFERENCE

Das Gupta,V.; Stewart,K.R. Quantitation of carbenicillin disodium, cefazolin sodium, cephalothin sodium, nafcillin sodium, and ticarcillin disodium by high-pressure liquid chromatography, *J.Pharm.Sci.*, **1980**, *69*, 1264–1267.

SAMPLE

Matrix: reaction mixtures
Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: MeCN:5.5 mM sodium octanesulfonate + 20 mM trisodium citrate dihydrate adjusted to pH 3 with concentrated HCl 28:72
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM

Retention time: 7.2
Limit of detection: 500 ng/mL

REFERENCE

Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:90 mM perchloric acid 13.5:86.5. B was MeCN:300 mM perchloric acid 45:55. A:B from 100:0 to 0:100 over 7 min, maintain at 0:100.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: 11

OTHER SUBSTANCES

Simultaneous: 7-aminocephalosproranic acid, cefadroxil, cefazolin, cefotaxime, cephalexin, cephaloridine, cephalosporin C, cephapirin, D-hydroxyphenylglycine

REFERENCE

Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE

Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18

Mobile phase: MeCN:10 mM ammonium acetate 22:78
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 240

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Selective analysis of mutual displacement effects at the primary binding sites of phenoxymethylpenicillin and cephalothin bindings to human serum albumin, *J.Pharmacobiodyn.*, **1992**, *15*, 91–97.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 220 × 4.6 Spheri 5 ODS-224
Mobile phase: 100 mM sodium dodecyl sulfate, pH 3.00
Flow rate: 1
Injection volume: 10
Detector: UV 260

CHROMATOGRAM

Retention time: 4.5

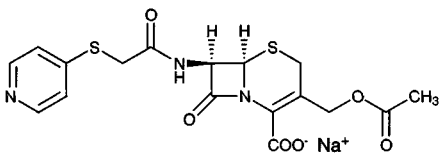
OTHER SUBSTANCES

Simultaneous: cefazolin, cephaloridine, cephalexin, cephradine, 7-aminocephalorospanic acid, 7-aminodesacetoxycephalosporanic acid

REFERENCE

Garcia Pinto,C.; Pérez Pavón,J.L.; Moreno Cordero,B. Micellar liquid chromatography of zwitterions: Retention mechanism of cephalosporins, *Analyst*, **1995**, *120*, 53–62.

Cephapirin sodium



Molecular formula: $C_{17}H_{16}N_3NaO_6S_2$
Molecular weight: 445.5
CAS Registry No.: 24356-60-3, 21595-23-7 (cephapirin)
Merck Index: 2030
Lednicer No.: 2 441

SAMPLE

Matrix: blood
Sample preparation: Prepare an anion-exchange SPE cartridge in a 6 mL syringe barrel with a filter paper disc in the bottom. Pack with DEAE-A-25 Sephadex in PBS to a bed volume of 3 mL, wash with PBS, place filter paper on top. 500 μL Serum + 10 μL 1 mg/mL cefoxitin, add to SPE cartridge, add 500 μL PBS to SPE cartridge, wash with 4 mL PBS, elute with 5 mL 1 M NaCl, inject a 100 μL aliquot of the eluate. (PBS was 8 g NaCl, 1.15 g Na_2HPO_4, 0.2 g KCl, and 0.2 g KH_2PO_4 in 1 L water, pH 7.2.)

HPLC VARIABLES

Column: 300 × 4 10 μm octadecylsilane
Mobile phase: MeCN:buffer 13:87 (Buffer was water adjusted to pH 2.8 with acetic acid, about 1.5 mL/min.)
Flow rate: 1.5
Injection volume: 100
Detector: UV 270

CHROMATOGRAM

Retention time: 3.7
Internal standard: cefoxitin (7.8)
Limit of quantitation: 1000 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, cefotaxime
Noninterfering: amikacin, amphotericin B, azathioprine, carbenicillin, chloral hydrate, cimetidine, dopamine, fluphenazine, furosemide, hydrochlorothiazide, insulin, levothyroxine, methylprednisolone, nitroglycerin, oxacillin, prednisone, procainamide, sulfamethoxazole, tolazamide, tolbutamide, triamterene, trimethoprim

KEY WORDS

serum; SPE

REFERENCE

Fasching,C.E.; Peterson,L.R. Anion-exchange extraction of cephapirin, cefotaxime, and cefoxitin from serum for liquid chromatography, *Antimicrob.Agents Chemother.*, **1982**, *21*, 628–633.

SAMPLE

Matrix: blood
Sample preparation: Mix serum with an equal volume of 250 μg/mL 4'-nitroacetanilide in MeCN:MeOH 90:10, mix, let stand at room temperature for 10 min, mix, centrifuge at 12800 g for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
Flow rate: 3

Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 6.7
Internal standard: 4'-nitroacetanilide (12.4)
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Extracted: cefamandole, cefazolin, cefotaxime, cefoxitin, chloramphenicol
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cephalexin, cephalothin, cimetidine, miconazole, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

KEY WORDS
serum

REFERENCE
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

SAMPLE
Matrix: blood
Sample preparation: 300 μL Plasma + 300 μL IS in ice-cold MeOH:100 mM pH 5.2 sodium acetate 70:30, vortex for 30 s, let stand at -20° for 10 min, centrifuge at 1500 g for 10 min, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 10 μm C18
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:100 mM sodium acetate 18.24:0.76:81, pH 5.2
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 4
Internal standard: cefoperazone (5.5)
Limit of detection: 500 ng/mL

KEY WORDS
plasma

REFERENCE
Signs,S.A.; File,T.M.; Tan,J.S. High-pressure liquid chromatographic method for analysis of cephalosporins, *Antimicrob.Agents Chemother.*, **1984**, *26*, 652–655.

SAMPLE
Matrix: blood, milk
Sample preparation: 500 μL Milk or serum + 500 μL MeCN:MeOH:water 40:20:40, vortex for 10-15 s, centrifuge through an Amicon Centricon-10 filter (10000 Dalton cut-off) at 3000 g for 30 min, inject a 10-40 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 220 × 2.1 5 μm Spheri-5 phenyl
Mobile phase: MeCN:MeOH:0.1% 85% phosphoric acid containing 5 mM sodium do decanesulfonate 20:5:75

Column temperature: 40
Flow rate: 0.4-0.5
Injection volume: 10-40
Detector: UV 291

CHROMATOGRAM
Retention time: 5.7
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; cow; ultrafiltrate

REFERENCE
Tyczkowska,K.L.; Voyksner,R.D.; Aronson,A.L. Development of an analytical method for cephapirin and its metabolite in bovine milk and serum by liquid chromatography with UV-VIS detection and confirmation by thermospray mass spectometry, *J.Vet.Pharmacol.Ther.*, **1991**, *14*, 51–60.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in mobile phase at a concentration of 200 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: DMF:acetic acid:45% KOH (w/w):water 5:0.2:0.1:94.7
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 13.3
Internal standard: acetanilide (18.5)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; stability-indicating

REFERENCE
MacNeil,L.; Rice,J.J.; Muhammad,N.; Lauback,R.G. Stability-indicating liquid chromatographic determination of cephapirin, desacetyl cephapirin and cephapirin lactone in sodium cephapirin bulk and injectable formulations, *J.Chromatogr.*, **1986**, *361*, 285–290.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Dissolve in water to a concentration of 20 μg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:water:acetic acid 30:70:0.1
Flow rate: 1
Injection volume: 20

Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Limit of quantitation: 980 ng/mL

OTHER SUBSTANCES
Simultaneous: impurities, cefadroxil, ceftizoxime, cefaclor, cefotaxime, cephalexin, cefazolin, cefoxitin, cephradine, cefoperazone, cefamandole, cephalothin, cefamandole nafate

REFERENCE
Ting,S. Reverse-phase liquid chromatographic analysis of cephalosporins, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 1123–1130.

SAMPLE
Matrix: milk
Sample preparation: Condition a Sep-Pak SPE cartridge with 3 mL MeOH and 5 mL water. 5 mL Milk + 10 mL 10 mM sodium acetate, mix, add to SPE cartridge, rinse in with 3 mL water, wash with 10 mL water, wash with 5 mL dichloromethane, dry with air for 1 min, add 500 μL mobile phase, elute with 1 mL MeCN:MeOH 75:25, evaporate eluate under reduced pressure until only a trace of liquid remains, make up to 500 μL with mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: 30 mm long Spheri-10 RP-18
Column: 150 × 4.6 5 μm Ultrasphere-ODS
Mobile phase: MeCN:MeOH:10 mM sodium acetate 112.5:37.5:850 (After cephapirin has eluted inject 500 μL MeCN:MeOH 75:25 to remove late-eluting compounds, allow 6 min for column to re-equilibrate. Wash column with 50 mL MeCN:MeOH 75:25 at end of each day.)
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 5.1
Limit of quantitation: 10 ppb

OTHER SUBSTANCES
Simultaneous: ampicillin, cloxacillin, penicillin G
Noninterfering: ceftiofur

KEY WORDS
cow; SPE

REFERENCE
MacIntosh,A.I. Liquid chromatographic determination of cephapirin residues in milk, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 880–882.

SAMPLE
Matrix: milk
Sample preparation: 10 mL Milk + 2 mL 200 mM tetraethylammonium chloride, stir, slowly add 38 mL MeCN over 30 s, let stand for 5 min, decant the supernatant through a plug of glass wool. 40 mL Filtrate + 1 mL water, evaporate under reduced pressure to 1-2 mL, make up to 4 mL with water, filter (0.45 μm polyvinylidene difluoride). Inject 2 mL into an LC system (150 × 4.6 5 μm Supelcosil LC-18; MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 40:60 over 27 min, to 0:100 over 1 min; 1 mL/min; UV 210 and 295), collect

a 1.5 mL fraction at retention time for cephapirin (18 min), evaporate to 1 mL, inject a 200 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 35:65 (Buffer was 15 mM phosphoric acid and 7.5 mM sodium dodecyl sulfate.)
Flow rate: 1
Injection volume: 200
Detector: UV 290

CHROMATOGRAM

Limit of quantitation: 2-5 ppb

OTHER SUBSTANCES

Also analyzed: ampicillin, amoxicillin, penicillin G, ceftiofur, penicillin V, cloxacillin

KEY WORDS

cow

REFERENCE

Moats,W.A.; Harik-Khan,R. Liquid chromatographic determination of β-lactam antibiotics in milk: A multiresidue approach, *J.AOAC Int.*, **1995**, *78*, 49–54.

SAMPLE

Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES

Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM

Retention time: 8.78

OTHER SUBSTANCES

Extracted: ampicillin, ceftiofur, cloxacillin, dicloxacillin, nafcillin, oxacillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS

SPE

REFERENCE

Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 19.8 min), evaporate to <1 mL under reduced pressure, add 200 μL 10 mM KH_2PO_4 containing 10 mM phosphoric acid and 10 mM sodium decanesulfonate, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 35:65 (Buffer was 15 mM phosphoric acid containing 7.5 mM sodium dodecyl sulfate.)
Flow rate: 1
Injection volume: 200
Detector: UV 290

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 4 μm Micropak SPC-18 C18
Mobile phase: Gradient. MeCN:10 mM orthophosphoric acid and 10 mM tetramethylammonium chloride from 15:85 to 60:40 over 20 min
Flow rate: 1
Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: amoxicillin, ampicillin

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100

g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)
Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 6.3

OTHER SUBSTANCES
Simultaneous: carbenicillin, cloxacillin, dicloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 OmniPac PCX-500 (Dionex)
Mobile phase: Gradient. A was MeCN:90 mM perchloric acid 13.5:86.5. B was MeCN:300 mM perchloric acid 45:55. A:B from 100:0 to 0:100 over 7 min, maintain at 0:100.
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: 7-aminocephalosproranic acid, cefadroxil, cefazolin, cefotaxime, cephalexin, cephaloridine, cephalosporin C, cephalothin, D-hydroxyphenylglycine

REFERENCE
Slingsby,R.W.; Rey,M. Determination of pharmaceuticals by multi-phase chromatography: Combined reversed phase and ion exchange in one column, *J.Liq.Chromatogr.*, **1990**, *13*, 107–134.

SAMPLE
Matrix: solutions

Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 20:80
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 260

OTHER SUBSTANCES

Also analyzed: cefamandole

REFERENCE

Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.